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Stimulation of Protective Antibodies against Type Ia and Ib Group B Streptococci by a Type Ia Polysaccharide-Tetanus Toxoid Conjugate Vaccine†

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Antisera elicited by type Ia group B streptococci (GBS) contain antibodies that react with both type Ia and type Ib strains. Previous studies suggested that antibodies elicited by type Ia organisms recognized a carbohydrate antigen or epitope common to Ia and Ib strains. We now report the synthesis and immunogenicity testing of a type Ia polysaccharide-tetanus toxoid (Ia-TT) conjugate vaccine. Ia-TT elicited type Ia polysaccharide-specific immunoglobulin G antibodies in all three of the rabbits inoculated. In competitive enzyme-linked immunosorbent assay, these antibodies reacted with high affinity to type Ia polysaccharide and with lower affinity to the structurally related GBS type Ib polysaccharide. Despite the lower binding affinity of the Ia-TT-induced antibodies for the type Ib polysaccharide, Ia-TT antiserum opsonized not only type Ia GBS but also type Ib GBS for killing by human blood leukocytes. Ia-TT antiserum was also evaluated in a mouse model designed to test the efficacy of maternal antibodies in protecting neonates against GBS infection. Pups born to dams that had received Ia-TT antiserum were protected against lethal challenge with either type Ia or Ib GBS. These studies using a polysaccharide-protein conjugate as an immunogen support the view that the carbohydrate immunodeterminant recognized on Ib strains by Ia antisera is a common epitope contained within the structurally related Ia and Ib capsular polysaccharides. Although antibodies elicited by Ia-TT had protective activity against both Ia and Ib strains, these antibodies reacted with lower affinity to Ib than to Ia polysaccharide.

During the 1970s, group B streptococci (GBS) surpassed *Escherichia coli* as the leading cause of bacterial infection among neonates in the United States (2). Organisms of capsular types Ia and Ib together account for 40 to 50% of cases of early-onset GBS disease (2). The type Ia and Ib capsular polysaccharides have chemically similar structures (12, 16): each is a polymer composed of pentasaccharide repeating units of galactose, glucose, *N*-acetylglucosamine, and *N*-acetylneuraminic (sialic) acid in a 2:1:1:1 molar ratio (Fig. 1). The sole difference between these polysaccharides is the bond between the side chain galactose and *N*-acetylglucosamine residues: these sugars are β(1→4)-linked in type Ia and β(1→3)-linked in type Ib (Fig. 1). Although the two polysaccharides are structural isomers, they are antigenically distinct; that is, antisera raised to type Ia organisms react strongly with homologous (type Ia) polysaccharide and to a much lesser degree with heterologous (type Ib) polysaccharide (16). However, Lancefield found that rabbit antisera raised to either type Ia or type Ib GBS protected mice against homologous and heterologous challenge (16). Lancefield named the putative cross-protective immunogenic determinant common to these two GBS serotypes Iabc. Subsequent immunochemical analysis suggested that the cross-reactive epitope was the backbone of the repeating-unit polysaccharide common to the type Ia and Ib polysaccharides (21).

The protective activity of capsule-specific antibodies stimulated efforts to develop vaccines based on these antigens. Studies have shown a strong positive correlation in the level of GBS antibodies in paired maternal-cord blood samples (5, 7), suggesting that maternal immunization might prevent neonatal GBS infections. Unfortunately, GBS type Ia polysaccharide, like the type III polysaccharide, is poorly immunogenic in humans (4). The immunogenicity of type Ia polysaccharide was tested in healthy adults whose levels of type Ia-specific antibodies were measured before vaccination (4). The response was dependent on the immune status of the volunteer; all 24 adults with a preexisting serum antibody level above 3 μg/ml responded to the vaccine, whereas only 40% of 67 subjects with low preexisting antibody levels (≤3 μg/ml) responded (4). Since most adults have only low levels of naturally acquired Ia-specific antibodies and since a low level of type-specific antibodies correlates with susceptibility of the neonate to GBS infection (1, 7), a GBS type Ia vaccine with improved immunogenicity would be an important advance in preventing neonatal infections due to type Ia strains.

Lagergard et al. (15) reported that conjugation of GBS type III polysaccharide to tetanus toxoid (TT) enhanced the antibody response to the polysaccharide in mice. Utilizing another conjugation method, we improved the immunogenicity of GBS type III and II polysaccharides in rabbits (19,

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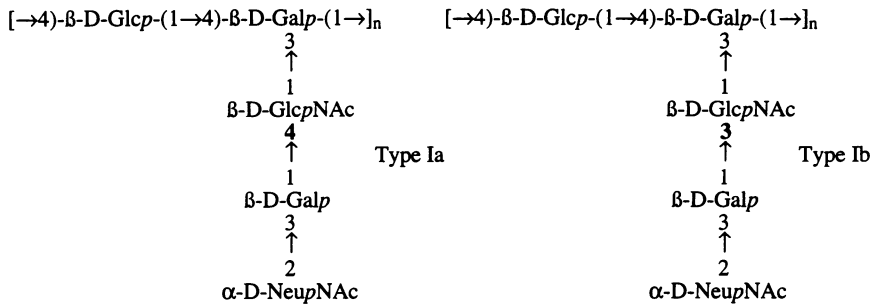

FIG. 1. Repeating-unit structures of GBS type Ia and Ib polysaccharides (12).

23). GBS polysaccharide-protein conjugates were prepared by covalent coupling of the polysaccharides to TT via reductive amination (19, 23). TT was directly conjugated to polysaccharide following periodate oxidation of a selected number of sialic acid residues on the polysaccharide (13, 19, 23). Because sialic acid is a terminal side chain saccharide on all GBS polysaccharides isolated thus far (22), the chemical approach we have used to couple GBS polysaccharides to proteins is theoretically feasible and would likely result in increased immunogenicity of all GBS capsular polysaccharides, including type Ia.

Here, we report the synthesis and immunogenicity testing of a GBS type Ia polysaccharide-TT (Ia-TT) conjugate vaccine. Antibodies elicited by Ia-TT were opsonic and protective against experimental infection with both Ia and Ib strains. Competitive binding studies using Ia-TT antisera indicated that the carbohydrate determinant recognized on type Ib strains is a common epitope shared by the Ia and Ib polysaccharides.

MATERIALS AND METHODS

Bacterial strains. Prototype GBS type Ia (strain 090) and type Ib (strain H36B) were originally obtained from the late Rebecca Lancefield, Rockefeller University, and were maintained as stock cultures at −80°C at the Channing Laboratory. Cells of strain 090 (16) were used in opsonophagocytic killing assays because this strain is highly encapsulated and is more resistant to killing by nonimmune serum, complement, and leukocytes than are less encapsulated strains (3). Strain 515 (obtained originally from Carol Baker, Baylor College of Medicine) was used in studies of passive protection because it is representative of clinical isolates with respect to the amount of capsular polysaccharide produced, susceptibility to in vitro opsonophagocytic killing, and lethality to animals (3).

Synthesis of GBS Ia-TT conjugate vaccine. Type Ia capsular polysaccharide, isolated from the highly encapsulated GBS type Ia strain 090, was purified and coupled to TT by methods detailed previously for the GBS type III polysaccharide (23). In brief, 15 mg of type Ia polysaccharide was dissolved in 2.0 ml of deionized water and oxidized by the addition of 150 μl of 10 mM $NaIO_4$. The oxidation reaction was allowed to proceed for 2 h at room temperature in the dark. The reaction was stopped by the addition to the mixture of 20 μl of ethylene glycol. Oxidized type Ia polysaccharide was then dialyzed against water and lyophilized to dryness. The proportion of sialic acid residues oxidized was determined by gas chromatography-mass spectrometry of trimethylsilyl derivatives as described elsewhere (23). TT (Institut Armand Frappier, Montreal, Canada) was purified to its monomeric form as reported previously (23). Oxidized type Ia polysaccharide (10 mg) was combined with 10 mg of TT in a total volume of 1.0 ml of 0.1 M sodium bicarbonate, pH 8.1. Sodium cyanoborohydride (20 mg) was added, and the mixture was incubated at 37°C for 8 days. The conjugate was separated from free TT by chromatography on a 1.6 by 85 cm column of Biogel A, 0.5M (Bio-Rad Laboratories, Richmond, Calif.), with phosphate-buffered saline (PBS) as the eluant. Void-volume fractions, monitored by A_{280}, were pooled, reduced with sodium borohydride, dialyzed against water, and lyophilized to dryness. Ia-TT vaccine was analyzed for its protein (17) and carbohydrate (9) contents, with bovine serum albumin and purified type Ia polysaccharide, respectively, used as standards.

Vaccination of rabbits. Groups of three New Zealand White rabbits (Millbrook Farms, Amherst, Mass.), each weighing ~3 kg, were vaccinated subcutaneously at several sites on the back with 50 μg of either GBS type Ia polysaccharide or Ia-TT. Both vaccines were emulsified with complete Freund's adjuvant. Booster doses of each vaccine (50 μg) mixed with incomplete Freund's adjuvant were administered by the same route 20 and 41 days after the initial dose. Blood was collected from each rabbit on days 0, 20, 34, 41, 55, and 70.

ELISA. Levels of type Ia-specific antibody were estimated by enzyme-linked immunosorbent assay (ELISA) on 96-well microtiter plates coated with 100 ng of type Ia polysaccharide–poly-L-lysine per well, as has been described elsewhere (11, 23). Carrier-specific antibody levels were also determined by ELISA using microtiter plates coated with 100 ng of monomeric TT per well (18). Antibodies were detected with goat anti-rabbit immunoglobulin G (IgG) (γ- and light-chain-specific) conjugated to alkaline phosphatase (Tago Inc., Burlingame, Calif.) at a dilution of 1:3,000. Antibody titers were recorded as the reciprocal dilution that resulted in an A_{405} of ≥0.3 when the appropriate reference serum (rabbit serum raised to whole cells of GBS type Ia strain 090 or to uncoupled TT), diluted 1:128,000 reached an A_{405} of 0.3.

Opsonophagocytic assay. The ability of vaccine-induced rabbit serum and of serum raised to whole cells of GBS type Ia strain 090 to promote killing of strain 090 or of type Ib GBS H36B by human blood leukocytes was tested in an assay of in vitro opsonophagocytosis as described previously (6). The complement source for all opsonophagocytic assays was 10% normal human serum previously absorbed with organisms of the same serotype as the target strain to remove specific antibodies. IgG and IgM isolated from pooled Ia-TT-induced rabbit serum (obtained on day 70 after primary immunization) were tested for their abilities to opsonize GBS type Ia. IgG- and IgM-containing serum

fractions were separated by affinity chromatography (18). In brief, a 0.5-ml volume of the pooled serum was loaded onto a column containing 1.0 ml of protein A-agarose (Pierce Chemical Co., Rockford, Ill.). The column was washed with PBS (pH 7.0), and unbound serum components were collected and pooled. Bound components were eluted from the column with 0.1 M glycine-HCl (pH 3.0) and pooled. After dialysis against PBS, the volume of each pool was adjusted to 5.0 ml with PBS. The samples were filtered sterile and stored at −20°C. Separation of IgM from IgG was confirmed by ELISA with the use of IgG (γ- and light-chain-specific [Tago Inc.]) and IgM (μ-chain-specific [Sera-Lab, Westbury, N.Y.]) alkaline phosphatase conjugates.

Passive protection of mice against GBS disease. Groups of 10 Swiss Webster outbred mice (Taconic, Germantown, N.Y.), weighing ~20 g each, were injected intraperitoneally with 0.2 ml of pooled serum obtained on day 70 from rabbits vaccinated with either native Ia polysaccharide or Ia-TT. Control mice (five per group) received pooled serum from unimmunized rabbits or rabbits vaccinated 70 days previously with uncoupled TT (18). On the following day, the mice were challenged intraperitoneally with 3.7×10^6 CFU of GBS type Ia strain 515 in 1.0 ml of Todd-Hewitt broth. The dose of strain 515 had been determined to be lethal for 90% of mice of similar strain and age. Mice surviving the bacterial challenge were counted daily for 3 days.

Neonatal mouse model of GBS infection. A previously developed neonatal mouse model of GBS infection (20) was used in this study to assess the protective efficacy of Ia-TT antisera in 1-day-old pups challenged with either type Ia strain 515 or type Ib strain H36B. Pregnant (18 to 20 days of gestation) Swiss Webster outbred mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were injected intraperitoneally with pooled rabbit serum raised to either Ia-TT (and obtained on day 70), whole cells of GBS type Ia strain 090, or whole cells of GBS type Ib strain H36B. Sera to whole GBS cells were made by the Lancefield method with use of formalin-killed cells (21). Control pregnant mice were injected with either preimmunization rabbit serum or rabbit serum raised to uncoupled TT (and obtained on day 70) (18). The challenge dose was administered intraperitoneally in a total of 0.05 ml of Todd-Hewitt broth with a tuberculin syringe and a 28-gauge needle. The number of pups that survived GBS infection was recorded 48 h after challenge.

Competitive ELISA. The serotype specificity and relative affinity of Ia-TT-induced serum were examined in a competitive ELISA in which purified homologous (type Ia) and heterologous (type Ib, II, and III) polysaccharides were used as inhibitors of antibody binding to Ia-coated ELISA wells. Serum raised in rabbits against whole cells of GBS type Ia strain 090 (21) was used in competition studies with type Ia and Ib polysaccharides.

Statistical analysis. Differences in the capacity of rabbit sera to protect neonatal mice against GBS disease were assessed by Fisher's exact test.

RESULTS

Biochemical characteristics of Ia-TT vaccine. The periodate-treated polysaccharide was analyzed by gas chromatography-mass spectrometry after reduction, methanolysis, and preparation of trimethylsilyl derivatives of the native or partially oxidized sialic acid residues. The signals representing the native sialic acid (C_9) derivative and the partially oxidized 8-carbon (C_8) derivative had relative abundances of

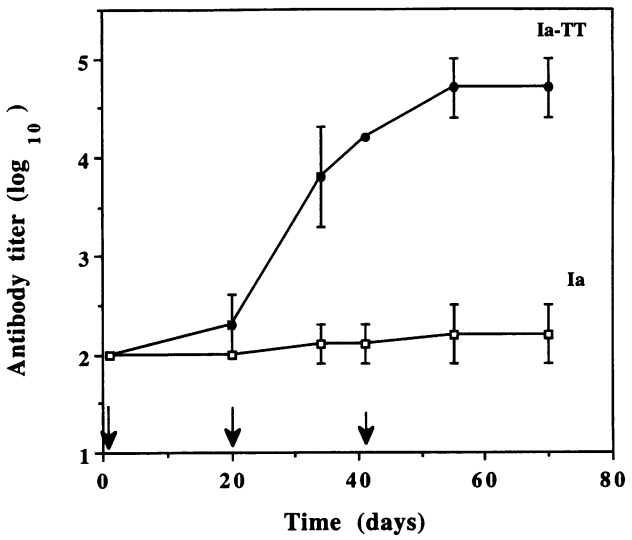

FIG. 2. Titers of type Ia polysaccharide-specific antibody in sera of rabbits vaccinated with either type Ia polysaccharide (Ia) or Ia-TT conjugate vaccine. Vaccine doses were administered on days 1, 20, and 41 (arrows). Results are presented as the means and standard deviations of type Ia-specific ELISA titers of serum samples from three rabbits per vaccine group.

90 and 10%, respectively. Thus, periodate oxidation of GBS type Ia polysaccharide resulted in the conversion of 10% of the polysaccharide's sialic acid residues to the 8-carbon analog of sialic acid, 5-acetamido-3,5-dideoxy-D-galacto-syloctulosonic acid. The modified sialic acid residues on the type Ia polysaccharide served as sites for coupling to TT by reductive amination. The purified Ia-TT conjugate vaccine was 12% (wt/wt) protein and 88% (wt/wt) carbohydrate.

Immunogenicity of GBS type Ia vaccines in rabbits. Vaccination of rabbits with a primary and booster dose of Ia-TT vaccine stimulated a rise in type Ia polysaccharide-specific antibody titer from 100 to 16,800 (Fig. 2). Type Ia polysaccharide-specific antibody levels rose still further following a third dose of Ia-TT vaccine. In contrast to the antibody response in rabbits vaccinated with Ia-TT vaccine, no type Ia polysaccharide-specific antibody response was evident in animals that received uncoupled, type Ia polysaccharide (Fig. 2). The protein carrier-specific response was also measured in animals that received the Ia-TT vaccine. Titers of TT-specific antibody peaked after animals received a primary and a booster dose of vaccine. After two doses of Ia-TT vaccine, levels of TT-specific antibody rose from a preimmunization level of 100 to 10,000 on day 34 (data not shown). Titers of TT-specific antibody did not increase further after a third dose of Ia-TT vaccine.

Serotype specificity of vaccine-induced antibodies. Homologous (type Ia) but not heterologous (type II or III) polysaccharide inhibited the binding of Ia-TT antisera to type Ia-coated wells in competitive ELISA (Fig. 3). The concentration of type Ia polysaccharide that resulted in 50% inhibition of Ia-TT antisera was 20 ng/ml, whereas no inhibition was detected with type II or type III polysaccharides, even at a concentration of 5 μg/ml (Fig. 3).

Antibodies raised in rabbits to either Ia-TT vaccine or whole cells of GBS type Ia recognized polysaccharide of both types Ia and Ib by competitive ELISA, although the 50% inhibitory concentration for the heterologous type Ib

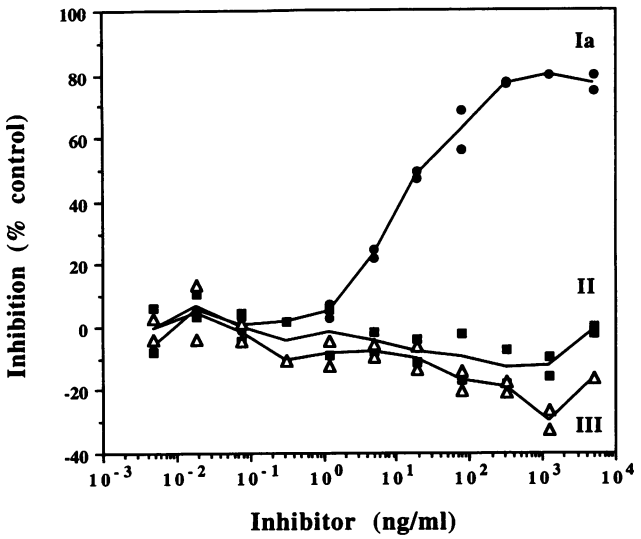

FIG. 3. Inhibition of the binding of GBS Ia-TT antiserum to type Ia polysaccharide by type Ia, II, and III polysaccharides. Increasing concentrations of homologous (type Ia) or heterologous (type II or III) polysaccharides were added to Ia polysaccharide-coated ELISA wells before the addition of Ia-TT antiserum. Datum points represent the percentage of inhibition in the presence of inhibitor compared with binding in control wells lacking inhibitor.

polysaccharide was 100-fold greater than that for the homologous type Ia polysaccharide (Fig. 4).

Functional activity of Ia-TT vaccine-induced rabbit serum. Both in vitro and in vivo assays were used to examine the functional capacity of rabbit antibodies raised against Ia-TT (Table 1). Serum obtained on day 70 from each of the three

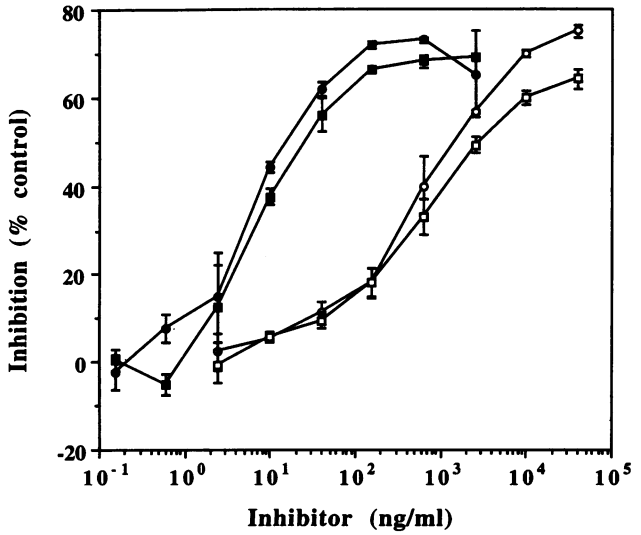

FIG. 4. Inhibition of the binding of GBS Ia-TT antiserum or whole-organism Ia antiserum to Ia polysaccharide by GBS type Ia or Ib polysaccharide. Increasing concentrations of type Ia polysaccharide (solid symbols) or type Ib polysaccharide (open symbols) were added to Ia polysaccharide-coated ELISA wells before the addition of Ia-TT antiserum (squares) or antiserum raised against whole type Ia organisms (circles). Datum points represent the percentage of inhibition in the presence of inhibitor compared with binding in control wells lacking inhibitor.

TABLE 1. In vitro opsonophagocytic killing of GBS type Ia strain 090

Serum (day)[a]	GBS $\log_{10}$ CFU at[b]:		Change ($\log_{10}$ CFU), 0–60 min
	0 min	60 min	
Ia-TT (0)	6.55	7.21	−0.66
Ia-TT, pooled (70)	6.63	4.91	1.72
Rabbit 1 Ia-TT (70)	6.47	4.76	1.71
Rabbit 2 Ia-TT (70)	6.46	4.71	1.75
Rabbit 3 Ia-TT (70)	6.70	4.81	1.89
Ia, pooled (70)	6.72	7.20	−0.48
TT (70)	6.84	7.20	−0.36

[a] Rabbit serum was added to assay mixtures at a final concentration of 1% (vol/vol). Sources of serum included rabbits vaccinated with Ia-TT conjugate, with uncoupled Ia polysaccharide (Ia), or with unconjugated TT. The day after immunization on which serum was obtained is given in parentheses.
[b] Values are the means of duplicate determinations.

rabbits vaccinated with Ia-TT vaccine promoted the in vitro killing of GBS type Ia strain 090 by human blood leukocytes. In the presence of these immune sera and with 10% human serum as a complement source, the number of type Ia cells in the assay was reduced by >1.7 $\log_{10}$. In contrast, serum from unimmunized animals or from animals vaccinated with uncoupled TT (18) or with uncoupled type Ia polysaccharide failed to promote killing of GBS type Ia cells.

Protein A-purified serum fractions containing IgG and IgM were also opsonically active against GBS type Ia strain 515. Affinity-purified IgG and IgM were added to different tubes in the opsonophagocytosis assay at 1% of the original concentration in serum, and after incubation with an antibody-free complement source and human leukocytes, the numbers of viable GBS type Ia cells were reduced by 1.5 $\log_{10}$ and 1.0 $\log_{10}$, respectively.

Immune rabbit serum shown to be opsonically active in vitro also protected adult mice against lethal challenge with type Ia GBS organisms. All of the 10 mice that received serum raised to Ia-TT vaccine survived challenge with a dose of GBS type Ia strain 515 previously shown to be lethal for 90% of a population of mice of similar age (Table 2). Ia-TT antiserum conferred significantly greater protection in this model than serum from unimmunized rabbits (P = 0.004), serum raised against native type Ia polysaccharide (P = 0.001), or serum raised against TT (P < 0.001).

Opsonic activities of rabbit sera raised to Ia-TT vaccine and to whole cells of GBS type Ia. Rabbit sera raised against Ia-TT vaccine and against cells of GBS type Ia promoted the in vitro killing by human blood leukocytes of GBS type Ia strain 090 and GBS type Ib strain H36B (Table 3). The

TABLE 2. Passive protection of adult mice against a lethal dose of GBS Ia strain 515

Rabbit serum[a]	No. of survivors/total no. of mice			Survival rate (%)
	Day 1	Day 2	Day 3	
Ia-TT	10/10	10/10	10/10	100[b]
Prevaccination	1/5	1/5	1/5	20
Ia	2/10	2/10	2/10	20
TT	1/5	0/5	0/5	0

[a] Pooled serum obtained from rabbits before vaccination or on day 70 after vaccination with native type Ia polysaccharide (Ia), Ia-TT, or uncoupled TT was administered to mice 24 h before bacterial challenge.
[b] P = 0.001 compared with Ia values, 0.004 compared with preimmunization values, and <0.001 compared with TT values.

TABLE 3. In vitro opsonophagocytic killing of GBS type Ia strain 090 and type Ib strain H36B

GBS target strain and serum[a]	Log_{10} bacteria killed by reciprocal serum dilution[b]			
	100	500	2,500	12,500
090 (type Ia)				
Ia-TT	2.21	1.78	1.99	−0.14
Ia whole cell	2.06	2.53	2.48	ND
H36B (type Ib)				
Ia-TT	0.96	1.34	0.31	0.00
Ia whole cell	ND	0.83	0.44	−0.07

[a] Sources of serum included rabbits vaccinated with Ia-TT obtained on day 70 or with whole cells of type Ia GBS (Ia whole cell).
[b] Values are the means of duplicate determinations and represent the difference in log_{10} CFU after 60 min of incubation. ND, not done.

number of cells of GBS type Ia strain 090 was reduced by ≥2 log_{10} when serum was used at final assay dilutions of 1:100, 1:500, and 1:2,500. The killing of GBS type Ib strain H36B by these sera, when used at a dilution of 1:500, was also significant (>0.8 log_{10}). Both sera had diminished opsonic activities against GBS type Ib cells at a dilution of 1:2,500.

Protection of neonatal mice against infection with GBS type Ia or type Ib. The major anticipated application of a GBS vaccine is its administration to childbearing women to elicit antibodies that will cross the placenta during pregnancy and protect the neonate after birth. To better mimic the human situation, we have developed a neonatal mouse protection model for GBS infection (20). IgG class antibodies are actively transported across the placenta both in mice (10) and in humans (14). In addition, the level of maternal IgG antibodies in the term fetus is closely correlated with the maternal serum level in both species. The protective efficacy of Ia-TT vaccine-induced antiserum against both type Ia and Ib infections was evaluated in this model (Table 4). Of pups whose mothers received a 1-ml dose of serum from rabbits vaccinated with Ia-TT, 92% survived a lethal dose of GBS type Ia strain 515 and 95% survived a lethal dose of GBS type Ib strain H36B. Survival rates of pups whose mothers received a 0.1-ml dose of antiserum were similar: 100% for type Ia and 89% for type Ib. Antiserum raised against whole cells of GBS type Ia or Ib provided 100% protection against a lethal challenge with GBS organisms of the homologous serotype. Antiserum to whole cells of GBS type Ia provided 70% protection against challenge with type Ib, and antiserum to whole cells of GBS type Ib provided 88% protection against challenge with type Ia. No pups whose mothers received serum from rabbits vaccinated with uncoupled TT survived (18), and 20% or less survived in groups whose mothers received prevaccination serum (Table 4).

TABLE 4. Protection of neonatal mice against GBS type Ia and Ib infection

Rabbit serum (dose)[a]	Response to GBS type Ia strain 515[b]		Response to GBS type Ib strain H36B[c]	
	No. of mice surviving/ total	Survival rate (%)	No. of mice surviving/ total	Survival rate (%)
Ia-TT vaccine (1 ml)	24/26	92[d]	40/42	95[d]
Ia-TT vaccine (0.1 ml)	14/14	100[d]	42/47	89[d]
TT	6/22	27	0/16	0

[a] Serum from vaccinated rabbits was administered to pregnant mice at 18 to 20 days gestation. One day after delivery the pups were challenged with an intraperitoneal injection of type Ia or Ib GBS.
[b] Each pup was challenged with 1×10^6 CFU of GBS type Ia.
[c] Each pup was challenged with 5×10^3 CFU of GBS type Ib.
[d] $P < 0.001$ compared with the group that received serum raised against TT.

Immunogenicity of Ia-TT with alum as adjuvant. To test the immunogenicity of Ia-TT with an adjuvant acceptable for use in humans, we vaccinated three rabbits with Ia-TT prepared similarly to the vaccine described above but mixed with alum (Alhydrogel; Superfos Biosector, Vedbaek, Denmark) instead of Freund's adjuvant. The vaccine was administered via the same route, dose, and schedule as described above. Peak antibody titers (day 54) to the Ia polysaccharide were 800, 1,600, and 6,400, for each of the three rabbits. Pooled serum from these animals, diluted 1:100, was opsonic for type Ia strain 090, mediating a 2.0 log_{10} decrease in CFU in the opsonophagocytic assay. These results indicated that, although antibody titers were lower with alum than with Freund's adjuvant, administration of Ia-TT with alum elicited functionally active, Ia polysaccharide-specific antibodies.

DISCUSSION

Over 50 years ago, Lancefield determined that type I GBS could be divided into two serologically distinct groups (16). She found antigenic differences between the polysaccharides of GBS type Ia and type Ib by using hot acid extracts of each type of strain and rabbit serum raised against formalin-killed cells as reagents for antigen-antibody precipitin tests (16). From these tests, she concluded "that type Ia and Ib are related but not identical" and upon further analysis determined that the bacterial component responsible for serotype specificity was polysaccharide in nature (16). A structural basis for the antigenic relatedness of the two antigens was suggested in 1983, when Jennings et al. (12) reported the repeating-unit structures of the type Ia and Ib polysaccharides. The type Ia and Ib polysaccharides (Fig. 1) are structural isomers, identical with respect to their glycose constituents, the molar ratio of these constituents, and the physical arrangement of the constituents except for a single glycosidic linkage of the repeating oligosaccharide unit (12).

When Lancefield first conducted experiments on strains of type I GBS, this organism was recognized as a cause of bovine mastitis and was considered to be a problem of the dairy industry (1). Since that time, GBS has become the leading bacterial cause of neonatal sepsis and meningitis among human infants (1, 2). Antibodies specific for the GBS capsular polysaccharides confer protection against GBS infection both in experimental animal models and in human neonates; however, native type Ia and III GBS polysaccharides are poor immunogens in humans who have low levels of preexisting GBS-specific antibodies (4). Although data on seroprevalence are limited, it appears that most adults have low or undetectable levels of GBS polysaccharide-specific antibodies. One study evaluating 291 obstetrical patients revealed that 87.6% had lower than protective levels (determined by this group to be 1 μg/ml) of naturally occurring type Ia-specific antibody (7).

Coupling of bacterial polysaccharides to an immunogenic protein carrier has been used successfully to increase the antibody response to the polysaccharide (reviewed in reference 8). We and others have coupled GBS polysaccharides to TT (15, 19, 23). We have used reductive amination to attach TT to GBS type III and II polysaccharides as a means of increasing the immunogenicity of these carbohydrate antigens (19, 23). Direct covalent conjugation of TT to a

portion of sialic acid residues on the GBS polysaccharides resulted in vaccines that were more immunogenic in rabbits than were uncoupled GBS polysaccharides (19, 23). Antisera raised in rabbits against GBS vaccines of this design not only opsonized GBS for in vitro killing by human blood leukocytes and complement but also protected mice against a GBS challenge (19, 23). In this study, we extended the application of the coupling technology to enhance the immunogenicity of GBS type Ia polysaccharide and to better define the antigenic relationship between GBS types Ia and Ib.

The type Ia polysaccharide-specific IgG response elicited in rabbits immunized with Ia-TT vaccine contrasted with the lack of response in rabbits vaccinated with uncoupled type Ia polysaccharide. The kinetics of the antibody response to the Ia-TT vaccine were similar to those seen in rabbits that received II-TT or III-TT vaccine: antibody levels were maximal after three doses (19, 23). Antibodies raised in rabbits against Ia-TT vaccine promoted the in vitro killing of GBS type Ia cells by human blood leukocytes in the presence of a complement and protected adult mice against a lethal dose of type Ia organisms. From these results, we conclude that the covalent coupling of GBS type Ia polysaccharide to TT was highly effective in improving the immunogenicity of the polysaccharide.

The specificity of vaccine-induced antibodies was examined by ELISA in competitive binding experiments. Binding patterns of Ia-specific antibodies elicited by Ia-TT were similar to those of antibodies elicited by whole type Ia organisms; this observation indicated that antigenic epitopes found on the polysaccharide component of the Ia-TT vaccine were similar to those found on whole cells of GBS type Ia. These epitopes were maintained in the Ia-TT vaccine despite the oxidation of a portion of the sialic acid residues on the Ia polysaccharide and the subsequent covalent attachment of TT to these residues. Antibodies elicited by Ia-TT vaccine did not react with type II or type III polysaccharide but did react, albeit with lower affinity, with type Ib polysaccharide. The complete inhibition of antibody binding to the Ia polysaccharide by the Ib polysaccharide indicated that the same antibodies bound to these two polysaccharides. This observation implies that the basis for cross-reactions of Ia antiserum with the Ib polysaccharide is not the stimulation of additional populations of antibodies with slightly different specificities (i.e., for the Ib polysaccharide) but rather the sharing of epitopes on the two antigens that are recognized by Ia-specific antibodies. These results support the view that the Iabc carbohydrate is a common epitope of the Ia and Ib polysaccharides rather than a separate surface molecule (16, 21). The shift to the right of the Ib binding curve relative to that for Ia (Fig. 4) reflects a lower overall affinity of binding of the Ib polysaccharide to Ia-specific antibodies. Thus, although the antibodies elicited by Ia-TT or by Ia organisms recognize the Ib polysaccharide, their higher affinity for Ia polysaccharide indicates that antibody binding is influenced substantially by the small difference in the repeating-unit structures of the two polysaccharides.

Rabbit antiserum raised against Ia-TT vaccine opsonized not only type Ia GBS but also type Ib organisms. In the opsonic tests, however, full activity against type Ia was seen at a serum dilution of 1:2,500, while full activity against Ib organisms was seen only at 1:500, suggesting that relative functional activity against the two serotypes parallels the relative binding affinities of the antibodies for the two polysaccharides in ELISA inhibition studies. These results supported the hypothesis that antibodies elicited by Ia-TT recognized the Ib polysaccharide but that antibody binding to the heterologous polysaccharide was of lower affinity than binding to the Ia polysaccharide. The fact that Ia-TT antiserum was opsonically active against Ib organisms suggested that Ia-TT antiserum might provide protection in vivo against both type Ia and Ib GBS. Protection against both serotypes by Ia-TT antiserum was demonstrated in the neonatal mouse model of GBS disease. Since the doses of antiserum used in the protection studies provided a high degree of protection against both serotypes, it is not possible, on the basis of these data, to determine whether activity was greater for Ia than Ib, as might be predicted on the basis of the relative binding affinities of the antibodies for the two polysaccharides. Studies with this model confirmed the functional activity of Ia-TT-induced antibodies against infection with both Ia and Ib GBS and showed that the antibodies could be transported across the placenta to achieve protective levels in the newborn.

These studies provide definitive evidence that cross-reactive and cross-protective antibodies can be elicited in rabbits by the GBS type Ia polysaccharide as part of a glycoconjugate vaccine. In addition, results obtained with the Ia-TT vaccine support the importance of the type Ia polysaccharide as a protective antigen, since the conjugate vaccine is acellular, containing only the polysaccharide capsule component of the GBS cell. Therefore, the protective antibodies that recognized both type Ia and Ib GBS elicited by the Ia-TT vaccine cannot have been elicited by other GBS antigens (e.g., surface proteins or group B carbohydrate) present in whole-cell vaccines (21). A GBS type Ia polysaccharide-protein conjugate of this design may be an effective vaccine for maternal immunization to protect human neonates against type Ia and possibly type Ib GBS disease.

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